Protein-apatite interactions in dentine

Boonstra, Willem Douwe

9.1 SUMMARY

In the last few decades in the whole western world a decline of enamel caries has been observed. This decrease is an immediate result of the application of fluorides, of improved eating habits and of a growing dental care. All these factors have resulted in a longer maintenance of the natural dentition by a large fraction of the population. In consequence of this, an increase of root caries as a result of gingival recession has come to the forefront. The roots of teeth consisting of dentine with a thin layer of cementum are easily attacked by acids produced by microorganisms in the plaque. The ultimate result is root (dentine) caries.

During caries the mineral component, mainly hydroxyapatite, is lost from the hard dental tissues. The amount of mineral loss and also the lesion depth is influenced by the presence of small amounts of "inhibitors". These inhibitors may originate from external sources (e.g. fluorides from toothpastes, salivary proteins) but can also be present in the tissue itself (internal inhibitors). No internal inhibitors are known in enamel. The results in this thesis show that internal inhibitors of proteineous nature play an important role in the root caries process. Both collagenous and non-collagenous proteins (NCP's) participate in the inhibition process.

At the start of the investigations only limited information was available on the influence of proteins on dentine demineralization, although the protein content of dentine is so high that such influence might be expected; about 20% of the total dentine weight is protein; 20 to 40 times more than in enamel. Therefore, the aims of this thesis have been:

(1) to study protein-apatite interactions in dentine
(2) to study the influence of proteins on dentine demineralization.

The literature survey in **chapter 2** is split up in two parts. In the first section attention is paid to the structure and chemical composition of dentine. Emphasis is put on the quantitative and qualitative information on the non-collagenous proteins (NCP's). There is only limited quantitative information available on NCP's and on the NCP distribution in the different dentine structures. In the second part, the background of protein-apatite interactions is discussed. The information, partly derived from hydroxyapatite chromatography, has also been shown to be applicable for protein adsorption onto enamel. Therefore, this information is also important in the study of protein-apatite interactions in dentine.

In **chapter 3**, the extraction of soluble dentine proteins (mostly NCP's) from bovine dentine slabs (so-called intact dentine) is described. More protein per gram released mineral is liberated when the demineralization is performed in 0.1M acetic acid (pH 5.0) than in 0.1M acetic acid (pH 4.5). No detectable amounts of collagen, the major part of the organic matrix, are released into the solution not even after one week of demineralization.

Chapter 4 describes the extraction of soluble dentine proteins as a function of the

pH of the demineralization / extraction solution. Furthermore the influence of the chemical composition of the buffer on protein extraction was investigated. This part of the study was performed with dentine powder so that a large surface area for protein release could be obtained with less material. It was discriminated between carbohydrate-containing and phosphate-containing NCP's. It appeared that the result of the NCP extraction is strongly dependent on the pH; the NCP's are poorly extractable between pH 2 and 4, much protein is released below pH 1 or above pH 5.5. Control experiments in which phosvitine adsorption onto dentine collagen was investigated, indicated that the poor extractability of the NCP's between the pH values 2 and 4 is probably a result of NCP-collagen (protein-protein) interactions. The phosphate-containing proteins in particular exhibit a high affinity for the dentine collagen. At pH 1 or lower, the repulsive forces between the positively charged NCP's and mineral surface (or collagen surface if mineral is lacking) are the cause for the high protein efflux. Above pH 5.5, the repulsive forces between the net negatively charged dentine components are the main cause for this high NCP release. Not only the pH value but also the calcium-binding properties of the extraction solution affect protein release. Calcium ion removal by calcium complexation or calcium salt formation gives rise to a increased protein release.

In **chapter 5** a four-step isolation of all the NCP's from bovine dentine and the adsorption of the resulting four different protein isolates to synthetic spheroidal HAP is described. The total amount of protein obtained after purification, is about 14 mg per gram dentine; the major part (50% or more) concerns phosphoproteins. The adsorption experiments have been performed at pH 6.7 and at pH 5 in a saturated calcium phosphate solution. The results show that all the NCP's exhibit a high affinity for the mineral hydroxyapatite (HAP) both at pH 6.7 and pH 5.0, except for the NCP's liberated after extraction of undemineralized dentine with 4M guanidine-HCl. Coverage of HAP by all the four NCP fractions increases with decreasing pH.

The influence on the demineralization rate *in vitro* (in aqueous solutions) by protein cross-linking in a 2% glutardialdehyde (GDA) solution with a pH value of about 3.5, is described in **chapter 6**. This treatment affecting dentine proteins in a small layer on the dentine surface, results in a decreased demineralization rate. This inhibition depends strongly on the calcium and phosphate concentration in solution at the start of the demineralization period; an increase in concentration of both ions initially increases the inhibition. The maximum inhibition as a result of a five-minute GDA treatment is almost 40%. The inhibition is most likely caused by:

(1) a reduction of the calcium and phosphate diffusion from the tissue into the solution, and/or
(2) a reduction of the diffusion of the demineralization inhibitors, most likely proteins, out of the tissue

In **chapter 7** the influence of the external proteins on intact dentine demineralization *in vitro* is investigated. The presence of proteins in solution during dentine

demineralization has little or no effect on dentine demineralization, in contrast to enamel demineralization as shown previously. This means for the *in vivo* situation that salivary proteins play most likely a less important role in the mechanism of dentine demineralization than these proteins do on enamel demineralization. The most probable explanations for this behaviour of dentine are (1) the proteins cannot adsorb onto the dentine crystallites due to steric hindrance during the diffusion process in dentine or during the adsorption process by dentine proteins already coating the crystallite surfaces completely or (2) the proteins in solution adsorb but do not contribute to the inhibition.

In the general discussion (**chapter 8**) the distribution of the main constituents of the dentine tissue over the various ultrastructural compartments is calculated and discussed. The calculations show that (with a number of approximations) about 75% of the NCP's are found <u>on</u> the mineralized collagen fibril surfaces in the intertubular dentine. The presence of NCP's <u>in</u> the collagen fibrils is unlikely. The calculated mineral surface in intertubular dentine of about 15 m^2 per gram dentine is almost equivalent to the total surface area per gram dentine needed for the adsorption of 75% of all the isolated NCP's onto HAP (chapter 5). Therefore, NCP's are most likely adsorbed as a monolayer onto the crystallites.